The Journal of International Advanced Otology

J Int Adv Otol 2017; 13(3): 308–12 • DOI: 10.5152/iao.2017.1674

Original Article

Changes in Proinflammatory Cytokines in the Cochlea in Relation to Hearing Thresholds in Noise-Exposed Rats

Hasan Hüseyin Arslan, Bülent Satar, Muhittin Serdar, Emine Yılmaz

Department of Otorhinolaryngology and Head and Neck Surgery, Centrium Hospital, İstanbul, Turkey (HHA)
Department of Otorhinolaryngology and Head and Neck Surgery, Gülhane Medical School, Ankara, Turkey (BS, EY)
Department of Biochemistry, Acıbadem University School of Medicine, İstanbul, Turkey (MS)

Cite this article as: Arslan HH, Satar B, Serdar M, Yılmaz E. Changes in Proinflammatory Cytokines in the Cochlea in Relation to Hearing Thresholds in Noise-Exposed Rats. J Int Adv 2017; 13: 308-12.

OBJECTIVE: Our aim was to investigate time effects in proinflammatory cytokines and the auditory brainstem response (ABR) thresholds of rat cochlea exposed to noise.

MATERIALS and METHODS: Twenty-one rats were divided into two groups: the control group and the noise group. As high as 115 dB sound pressure of white noise was administered to the noise group of 16 rats for 3 h a day for 10 days. This group was further split into four subgroups based on the timing of sacrifice: 3rd hour group, 12th hour group, 24th hour group, and 28th day group. ABR thresholds were measured in all the rats, after the noise exposure and right before being sacrificed. Proinflammatory cytokine levels (IL-6, IL-1β, and TNF-α) at the cochlea were measured.

RESULTS: We found a significant difference between the first ABR thresholds (5 dB nHL) and the post-exposure ABR thresholds in each group (25 dBnHL, 35 dBnHL, 15 dBnHL, and 17.50 dBnHL for the 3rd hour group, 12th hour group, 24th hour group, and 28th day group, respectively). The IL-1β levels in the 3rd hour group and 12th hour group were significantly higher than those in the control group and other noise subgroups. The TNF-α level in the 3rd hour group was significantly higher than that in the control group and other noise subgroups.

CONCLUSION: It seems reasonable to point out a direct correlation between the cytokine levels and hearing threshold levels after the noise exposure. This correlation was the highest for IL-1β. This result suggested a significant role of proinflammatory cytokines in hearing deterioration after noise exposure.

KEYWORDS: Noise-induced hearing loss, proinflammatory cytokines, noise

INTRODUCTION

It is well known that excessive noise exposure can cause hearing loss through different mechanisms [1]. Noise-triggered damage leads to impairment of the stereocilias of the hair cells, the loss of hair cells, and structural distortion at the spiral ligament and spiral ganglion [2, 3]. It was reported that hair cells become dysfunctional within 2 weeks after noise exposure, and an edema develops in the spiral ganglion neurons [4, 5]. Nevertheless, the molecular mechanisms of noise-induced hearing loss are still not fully understood. In the broadest sense, excessive noise causes direct mechanical trauma to cochlear structures and an increase in reactive nitrogen species and reactive oxygen species, which can then destroy the DNA and cell membranes [1].

The roles of the proinflammatory cytokines of the inner ear after the noise exposure have been enlightened in some studies [6-8]. The main reason for cochlear cell damage might be the local increase in proinflammatory cytokines because the amount of these molecules at the damaged cochlea increases within a day and induces a gathering of inflammatory cells. Furthermore, proinflammatory cytokines are secreted from some blood-originated inflammatory cells and neurons. A potential role in the second-line inflammatory effects of these molecules has been previously proposed [9]. However, the secretion mechanism of cytokines at the damaged cochlea is not yet fully clear. Therefore, measuring proinflammatory cytokines in the cochlea exposed to noise may shed light on the relationship between hearing level and cytokines in noise-induced hearing loss (NIHL). This study aimed to seek a correlation between hearing thresholds and the level of proinflammatory cytokines.

Corresponding Address: Hasan Hüseyin Arslan E-mail: hharslan@ymail.com
Submitted: 16.09.2015 **Revision received:** 14.06.2016 **Accepted:** 20.04.2016

MATERIALS and METHODS

Animals

The study protocol was approved by our institutional ethics committee (May 15, 2009/AR-2009/30). The study was performed in 21 male Wistar rats. Normal outer ear canal and ear drum was confirmed in all the rats. Normal hearing level (5 dB nHL) was confirmed in auditory brainstem response (ABR) testing prior to the noise administration in all the rats.

Groups

Twenty-one rats (42 ears) were grouped into two groups: the control group and the noise group, which was then subdivided into a number of subgroups. Table 1 presents the procedures administered to the rats.

Control group: Five rats (10 ears) were assigned to the control group. Initial ABR thresholds were recorded and then the rats were sacrificed. Cytokines (IL-6, IL-1β, and TNF-α) were measured in the removed cochlear soft tissues using ELISA.

Noise group: The noise group comprised 16 rats (32 ears) that were exposed to the predetermined noise level after the initial ABR test. ABR thresholds were recorded immediately after the exposure. A repeat ABR test was performed to detect the latest thresholds at predetermined time points based on the subgroups. The rats were then sacrificed, and the cytokines were measured in the removed cochlear soft tissues using ELISA. The 16 rats (32 ears) were equally divided into four subgroups based on the predetermined sacrifice time: 3rd hour group, 12th hour group, 24th hour group, and 28th day group.

ABR testing

All ABR tests were performed under general anesthesia. General anesthesia was obtained from 40 mg/kg im ketamine hydrochloride (Ketalar vial; Pfizer Co, Istanbul, Turkey) used in conjunction with 5 mg/kg im xylazine hydrochloride (Rompun vial; Bayer, Istanbul, Turkey) for ABR tests. Ipsilateral potentials were collected using needle electrodes. The ground electrode and reference electrode were placed on the vertex in the midline, and an active electrode was placed on the mastoid. Details of ABR testing are described in the literature [10]. As the second wave was the most robust activity, it was taken as a reference for the ABR threshold.

Noise exposure

All 16 rats (32 ears) of the noise group were subjected to free field white noise of 115 dB SPL (sound pressure level) for 3 h a day for 10 days. Details of the noise protocol were previously published [8].

Microdissection of the cochlear soft tissue and measurement of IL-6, IL-1β, and TNF-α levels

A lethal dose of thiopental sodium (IE Ulagay, Istanbul, Turkey) was injected for rat sacrifice. Procedures for the removal of the cochlear soft tissue and measurement of the IL-6, IL-1β, and TNF-α levels were previously described [10].

Statistical Analysis

Statistical Package for Social Sciences version 16.0 (SPSS Inc.; Chicago, IL, USA) was used for the statistical evaluation of the results from all 21 rats (42 ears). Pre-exposure and post-exposure ABR thresholds and cytokine levels were compared within the groups using the Wilcoxon test, and among groups using the Mann-Whitney U test. Significance was accepted at a p value of <0.05.

RESULTS

ABR Thresholds

All rats in the control and noise groups showed 5 dB nHL-ABR thresholds prior to the noise exposure. All rats from the noise group had hearing loss at certain levels, as confirmed by ABR tests performed right after the noise administration. A significant difference was noted between the initial (5 dB nHL) and the immediate post-exposure ABR thresholds in the noise subgroups (25 dB nHL, 35 dB nHL, 15 dB nHL, and 17.50 dB nHL for the 3rd hour group, 12th hour group, 24th hour group, and the 28th day group, respectively) (p<0.05, Wilcoxon test) (Figure 1). The latest ABR test performed after the specified times of each subgroup showed that three ears (7.5%) recovered to some extent (both ears of one rat and one ear of another rat) on day 28.

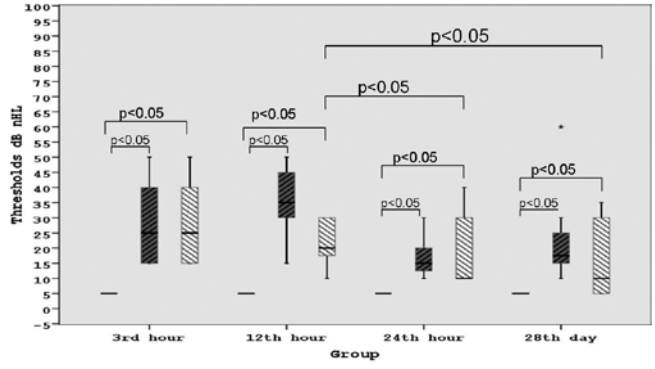

Figure 1. Changes in ABR thresholds after noise exposure (dashed lines: initial threshold, black boxes: immediate post-exposure threshold, striped boxes: latest threshold)

ABR: auditory brainstem response

Table 1. Procedures undergone by the rats

		First ABR	Noise exposure	ABR right after the noise	Latest ABR (at sacrifice time) and Cytokine analysis
Control group (n=5 rats/10 ears)		+			−/+
Noise group (n=16 rats/ 32 ears)	3rd hour group	+	+	+	−/+
	12th hour group	+	+	+	+/+
	24th hour group	+	+	+	+/+
	28th day group	+	+	+	+/+

ABR: auditory brainstem response

However, in the remaining 29 ears (90.6%), hearing thresholds were higher than those before the noise exposure. Comparison of all the latest thresholds yielded a significant difference (p<0.05, Mann-Whitney U test) between the 12th hour group (20 dB nHL) and the other two groups (10 dB nHL in the 24th hour group and 10 dB nHL in the 28th day group) and between the 24th hour (10 dB nHL) and the 28th day (10 dB nHL) groups (Figure 1). When we considered the latest ABR thresholds of each subgroup at the specified times, 15 dB-, 5 dB-, and 5 dB- increases relative to the pre-exposure thresholds were observed in the 12th hour group, 24th hour group, and 28th day group, respectively. Significant differences were noted between the latest thresholds and the initial thresholds (p<0.05, Wilcoxon test) (Figure 1). When considering only the post-exposure thresholds, the threshold of the 24th hour group was significantly lower than that of the 12th hour group (p<0.05). No other significant difference was determined among the other post-exposure thresholds (p>0.05, Mann-Whitney U test).

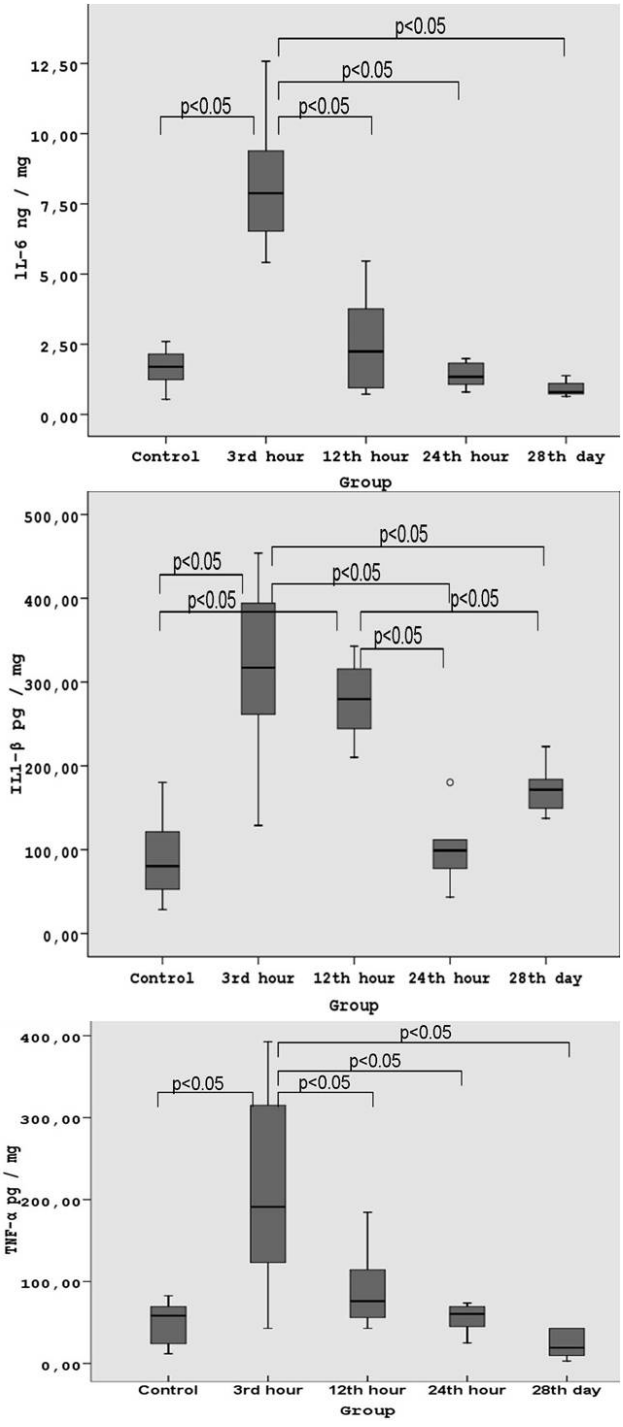

Figure 2. a-c. (a) Changes in IL-6 levels in control group and noise groups, IL-1β, and TNF-α levels, (b) changes in IL-1β levels in control group and noise groups, (c) changes in TNF-α levels in control group and noise groups

Cytokines in the Cochlear Soft Tissue

Figure 2 demonstrate the changes observed in the IL-6, IL-1β, and TNF-α levels among the groups. IL-6 level of the 3rd hour group (7.88 ng/mg) was significantly higher than that of the control group (1.69 ng/mg) and all other noise groups (2.24, 1.34, and 0.80 ng/mg, respectively) (p<0.05 Mann-Whitney U test) (Figure 2). No other significant difference was found among the other noise groups or between the control group and the other noise groups (p>0.05, Mann-Whitney U test) (Figure 2). IL-1β levels of the 3rd hour group (317.10 pg/mg) and the 12th hour group (279.64 pg/mg) were significantly higher than those of the control group (80.37 pg/mg) and the other noise groups (99.01, 171.48 pg/mg, respectively) (p<0.05, Mann-Whitney U test) (Figure 2). No other significant change was observed among the other noise groups or between the control group and the other noise groups (p>0.05, Mann-Whitney U test) (Figure 2). TNF-α level of the 3rd hour group (191.07 pg/mg) was significantly higher than those of the control group (58.28 pg/mg) and all other noise groups (76, 60.49, 19.21 pg/mg, respectively) (p<0.05, Mann-Whitney U test) (Figure 2). No other significant difference was detected among the other noise groups or between the control group and the other noise groups (p>0.05, Mann-Whitney U test) (Figure 2).

Correlation between Cytokine Levels and ABR Thresholds

A direct correlation was observed between the cytokine levels and hearing thresholds after the noise. This correlation was the highest for IL-1β (Table 2). This result suggested that the deterioration in hearing after noise exposure may be due to the inflammatory cytokines released from the cochlea.

DISCUSSION

Exposure to excessive noise can cause hearing loss by means of mechanical trauma and second-line trauma caused by new inflammatory processes [6]. One of the mechanisms of secondary damage might be a local increase in the inflammatory response and proinflammatory cytokines because these cytokines rise endogenously in the first 2 weeks after noise exposure, which in turn increases inflammatory cell infiltration [8, 11].

Table 2. Correlation between ABR threshold and cytokine levels

	IL-6	IL-1β	TNF-α	Latest ABR threshold
IL-6	X	0.589(**)	0.663(**)	0.326(**)
IL-1β	0.589(**)	X	0.678(**)	0.407(**)
TNF-α	0.663(**)	0.678(**)	X	0.288(*)
Latest ABR threshold	0.326(**)	0.407(**)	0.288(*)	X

ABR: auditory brainstem response

**Correlation at the 0.01 level is significant

Various researchers have reported the migration of inflammatory cells into the cochlea after damage, such as following ototoxic drug use, exposure to excessive noise, and surgical trauma [12, 13]. Satoh et al. [14] demonstrated that excessive infiltration of the inner ear by monocytes and macrophages after ototoxic injury caused cochlear degeneration and hearing loss. Although inflammatory cell infiltration of the cochlea after acoustic trauma is well known, the role of proinflammatory cytokines released by these cells in noise-induced hearing loss is still not fully clear. Measuring the changes in proinflammatory cytokines in relation to hearing thresholds can help us to understand the mechanism underlying hearing loss caused by acoustic trauma.

Fujioka et al. [13] found higher levels of IL-6 and IL-1β 3 h after the noise exposure, which returned to normal levels in 24 h. The difference in TNF-α levels was not significant. Our results are in line with other studies pointing out the expression of proinflammatory cytokines in noise-damaged cochleae [6, 13]. In the present study, we demonstrated the expression of IL-6, IL-1β, and TNF-α in the early phase of noise-induced cochlear damage. When considering changes in IL-6 in the timeline, IL-6 significantly increased at the 3rd hour group and its level at the 12th hour group significantly sloped down to a level that was still higher ($p<0.05$) than its level in the control group. From the 24th hour, its level was closer to baseline. IL-1β showed a drop at the 12th hour. At the 24th hour, it was very close to baseline. Then, interestingly, it made a second incline on the 28th day. The course of TNF-α in the timeline was similar to that of IL-6, where it showed a gradual decline from the 12th hour onwards.

Another important result of the study is the correlation between ABR thresholds and cytokine levels. The highest correlation of threshold was with IL-1β, followed by IL-6 and TNF-α. All cytokines increased within the first 3 h of the noise exposure when the hearing loss peaked. Then, the cytokine levels got closer to baseline at the 24th hour after the exposure. At this time, the threshold improved. This result implies a possible role of cytokines in secondary damage to the cochlea in NIHL. Interestingly, we also observed a significant correlation among the levels of three cytokines after the noise. Particularly in the early period of the noise, a significant increase was observed in all cytokine levels, whereas all the three cytokines decreased from the 12th hour onwards. Previous studies reported an increased expression of IL-6 by lateral wall fibrocytes after IL-1β and TNF-α stimulation [13, 15].

An emphasis on the roles of the cytokines may enable us to understand changes in hearing in association with changes in cytokine levels. TNF-α accumulates proinflammatory cells in the cochlea [11]. It was experimentally shown that the inhibition of TNF-α and suppression of inflammatory cells reduced hearing loss [4]. IL-6 upregulates some apoptotic genes and thereby, oxidative stress [16]. So et al. [17] reported the induction of IL-6 in wider cochlear damage, such as from noise exposure and cisplatin treatment. Fujioka et al. [13] showed that IL-6 was released in the early phase of acoustic trauma, and IL-6 was a major inducer of acute cochlear damage. They also showed an improvement in hearing when the IL-6 signal was inhibited.

Considering these data, we propose that the inhibition of cytokine secretion as early as possible is beneficial for precluding further damage. Steroids with anti-TNF-α effects are the most preferred drugs for the recovery of hearing in acoustic trauma [18]. A drug with the effect of an anti-IL-6 receptor antibody was experimentally shown to be beneficial in noise-exposed mice [12].

CONCLUSION

To conclude, this study showed that proinflammatory cytokines play an important role in hearing loss after noise exposure. Moreover, the hearing loss seemed to involve a direct correlation between the cytokine levels and hearing thresholds levels after noise exposure. This correlation was the highest for IL-1β. Detailed knowledge of the specific roles of these cytokines will allow us not only to better understand the mechanism of hearing loss after noise exposure but also to develop new therapeutic approaches, such as the blockade of proinflammatory cytokines.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gülhane Medical School (Approval Date: 15.05.2009/Approval No: AR 2009/30).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - H.H.A., B.S.; Design - H.H.A., B.S.; Supervision - B.S., M.S.; Resources - H.H.A., E.Y.; Materials - H.H.A.; Data Collection and/or Processing - H.H.A., E.Y., B.S.; Analysis and/or Interpretation - H.H.A., B.S., M.S.; Literature Search - H.H.A.; Writing Manuscript - H.H.A.; Critical Review - H.H.A., B.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Yamashita D, Jiang HY, Schacht J, Miller JM. Delayed production of free radicals following noise exposure. Brain Res 2004; 1019: 201-9. [CrossRef]
2. Liberman MC, Dodds LW. Acute ultrastructural changes in acoustic trauma: serial-section reconstruction of stereocilia and cuticular plates. Hear Res 1987; 26: 45-64. [CrossRef]
3. Gao WY, Ding DL, Zheng XY, Ruan FM, Liu YJ. A comparison of changes in the stereocilia between temporary and permanent hearing losses in acoustic trauma. Hear Res 1992; 62: 27-41. [CrossRef]
4. Puel JL, Ruel J, Gervais d'Aldin C, Pujol R. Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. Neuroreport 1998; 9: 2109-14. [CrossRef]
5. Juhn SK, Rybak LP. Labyrinthine barriers and cochlear homeostasis. Acta Otolaryngol 1981; 91: 529-34. [CrossRef]
6. Hirose K, Discolo CM, Keasler JR, Ransohoff R. Mononuclear phagocytes migrate into the murine cochlea after acoustic trauma. J Comp Neurol 2005; 489: 180-94. [CrossRef]
7. Keithley EM, Wang X, Barkdull GC. Tumor necrosis factor alpha can induce recruitment of inflammatory cells to the cochlea. Otol Neurotol 2008; 29: 854-9. [CrossRef]
8. Ladrech S, Wang J, Simonneau L, Puel JL, Lenoir M. Macrophage contribution to the response of the rat organ of Corti to amikacin. J Neurosci Res 2007; 85: 1970-9. [CrossRef]
9. Lamm K, Arnold W. Successful treatment of noise-induced cochlear ischemia, hypoxia, and hearing loss. Ann N Y Acad Sci 1999; 884: 233-48. [CrossRef]
10. Arslan HH, Satar B, Serdar MA, Ozler M, Yilmaz E. Effects of Hyperbaric Oxygen and Dexamethasone on Proinflammatory Cytokines of Rat Cochlea in Noise-Induced Hearing Loss. Otol Neurotol 2012; 33: 1672-8. [CrossRef]
11. Okano T, Nakagawa T, Kita T, Kada S, Yoshimoto M, Nakahata T, Ito J. Bone marrow-derived cells expressing Iba1 are constitutively present as resident tissue macrophages in the mouse cochlea. J Neurosci Res 2008; 86: 1758-67. [CrossRef]

12. Wakabayashi K, Fujioka M, Kanzaki S, Okano HJ, Shibata S, Yamashita D, et al. Blockade of inter- leukinY6 signaling suppressed cochlear inflammatory response and improved hearing impairment in noise-damaged mice cochlea. Neurosci Res 2010; 66: 345-52. [CrossRef]
13. Fujioka M, Kanzaki S, Okano HJ, Masuda M, Ogawa K, Okano H. Proinflammatory cytokines expression in noise-induced damaged cochlea. J Neurosci Res 2006; 83: 575-83. [CrossRef]
14. Satoh H, Firestein GS, Billings PB, Harris JP, Keithley EM. Tumor necrosis factor- alpha, an initiator, and etanercept, an inhibitor of cochlear inflammation. Laryngoscope 2002; 112: 1627-34. [CrossRef]
15. Ichimiya I, Yoshida K, Suzuki M, Mogi G. Expression of adhesion molecules by cultured spiral ligament fibrocytes stimulated with proinflammatory cytokines. Ann Otol Rhinol Laryngol 2003; 112: 722-8. [CrossRef]
16. Lin MT, Juan CY, Chang KJ, Chen WJ, Kuo ML. IL-6 inhibits apoptosis and retains oxidative DNA lesions in human gastric cancer AGS cells through upregulation of anti-apoptotic gene mcl-1. Carcinogenesis 2001; 22: 1947-53. [CrossRef]
17. So H, Kim H, Lee JH, Park C, Kim Y, Kim E, et al. Cisplatin cytotoxicity of auditory cellsrequires secretions of proinflammatory cytokines via activation of ERK and NF kappaB. J Assoc Res Otolaryngol 2007; 8: 338-55. [CrossRef]
18. Maeda K, Yoshida K, Ichimiya I, Suzuki M. Dexamethasone inhibits tumor necrosis factor-alpha-induced cytokine secretion from spiral ligament fibrocytes. Hear Res 2005; 202: 154-60. [CrossRef]